VOLUME DEPENDENT EFFECT OF HYPOTONIC TREATMENT ON GOOD SPREADING OF FISH CHROMOSOMES

by

Fausto FORESTI (1), Catherine OZOUF-COSTAZ (2) and Petr RAB (3)

ABSTRACT. - The authors have tested the effects of different volumes of hypotonic solution combined with two different fixative treatments on kidney cells of two fish species, *Silurus glanis* and *Scardinius erythrophthalmus*. They conclude that chromosome spreading is highly dependent on the volume of the hypotonic solution regardless of prefixation procedure.

RÉSUMÉ. - Une série de traitements hypotoniques a été appliquée à des cellules de rein de deux espèces de poissons, *Silurus glanis* et *Scardinius erythrophthalmus*, combinée avec deux systèmes différents de préfixation. Les résultats indiquent clairement qu'un bon étalement et une dispersion correcte des chromosomes sont liés au volume de la solution hypotonique, indépendamment du système de préfixation.

Key-words. - Fish cytogenetics, Hypotonic treatment, Prefixation.

Although the physical basis of chromosome preparations from live animal cells are quite well understood (Holmquist and Motara, 1987) and the basic principles are the same among all vertebrate groups (i.e. isolation of dividing cells, their hypotonization and subsequent fixation), detailed procedures can significantly differ among and also within these vertebrate groups. Fish cytogenetics, although now well equipped with powerful methods (Gold *et al.*, 1990; Thorgaard and Disney, 1990; Klinkhardt, 1991) is still far to provide regularly satisfactory results as compared to those obtained with mammals. Besides reaching a good mitotic rate, the most critical step in fish chromosome preparations is the correct duration of the hypotonic treatment, that allows a good spreading of metaphasic cells on slide preparations: too short hypotonic treatments provoke a bad spreading of chromosomes, while too long ones can cause chromosome losses. However the exact determination of the duration and temperature of the hypotonic treatment is difficult because it is commonly accepted that such optimal treatment can depend not only on a given fish group (freshwater, marine, migratory, cold water, temperate, tropical) but also on season, sexual maturity, age, feeding and health conditions, and undoubtedly on individual response to such treatment. To stop the swelling effects of hypotonic solution, one of us (F. Foresti) has adopted and modified prefixation step known in human cytogenetics in his protocol for fish chromosome preparations as follows: prepared cell suspension is hypotonized in 5 ml of 0.58% KCl solution for the estimated duration and temperature. Hypotonization is stopped by adding two drops of fixative (methanol, acetic acid 3:1), mix and let for five minutes, then 5 ml of fixative are

---

(1) IB/UNESP, Botucatu SP 18600, BRASIL.

(2) Muséum national d'Histoire naturelle, Laboratoire d'Ichtyologie générale et appliquée, 43 rue Cuvier, 75231 Paris cedex 05, FRANCE.

(3) Institute of Animal Physiology and Genetics, 277 21 Libechov, CZECHOSLOVAKIA.

Cybium 1992, 16(3): 209-212.

gently mixed with the cell suspension which is immediately centrifuged. Prefixation step is regularly used because it seems to increase the resistance of swelled cells to mechanical shaking.

While studying the chromosomes of several fish species using this protocol, but with smaller cell suspension volumes, we observed a factor other than duration of hypotonic treatment which significantly affected the quality of chromosome preparations. The present technical note describes the effects of the volume of hypotonic solution and prefixation procedure on good chromosome spreading.

MATERIAL AND METHODS

Six juveniles of *Silurus glanis* (Siluridae) and two specimens (one male, one female) of *Scardinius erythrophthalmus* (Cyprinidae) were used.

The experimental procedures were applied at room temperature as follows:

Silurus glanis. Cells of anterior kidney (maximum weight, 0.1 g) suspended in 2.5 ml of KCl solution; hypotonic treatments for respectively 40, 60 and 90 mn were applied; then 2 drops of fixative were added; after 5 mn, 2.5 ml of fixative were gently mixed with cell suspension before centrifugation followed by three additional changes of fixative solution.

Scardinius erythrophthalmus. Kidney cell suspension of the same animal was divided into several aliquots (each corresponding to about 0.1 g of tissue) in order to ensure that differences in the results are not due to individual responses. Duration of hypotonic treatment were the same for all procedures (40 mn).

1/ Same procedure as for *S. glanis*.

2/ 5 ml of hypotonized suspension prefixed **only** by two drops of fixative, **not** followed by adding 5 ml of fixative.

3/ 5 ml of hypotonized suspension prefixed by 2 drops of fixative, followed by adding 5 ml of fixative.

4/ 10 ml of hypotonized suspension prefixed by 4 drops of fixative only.

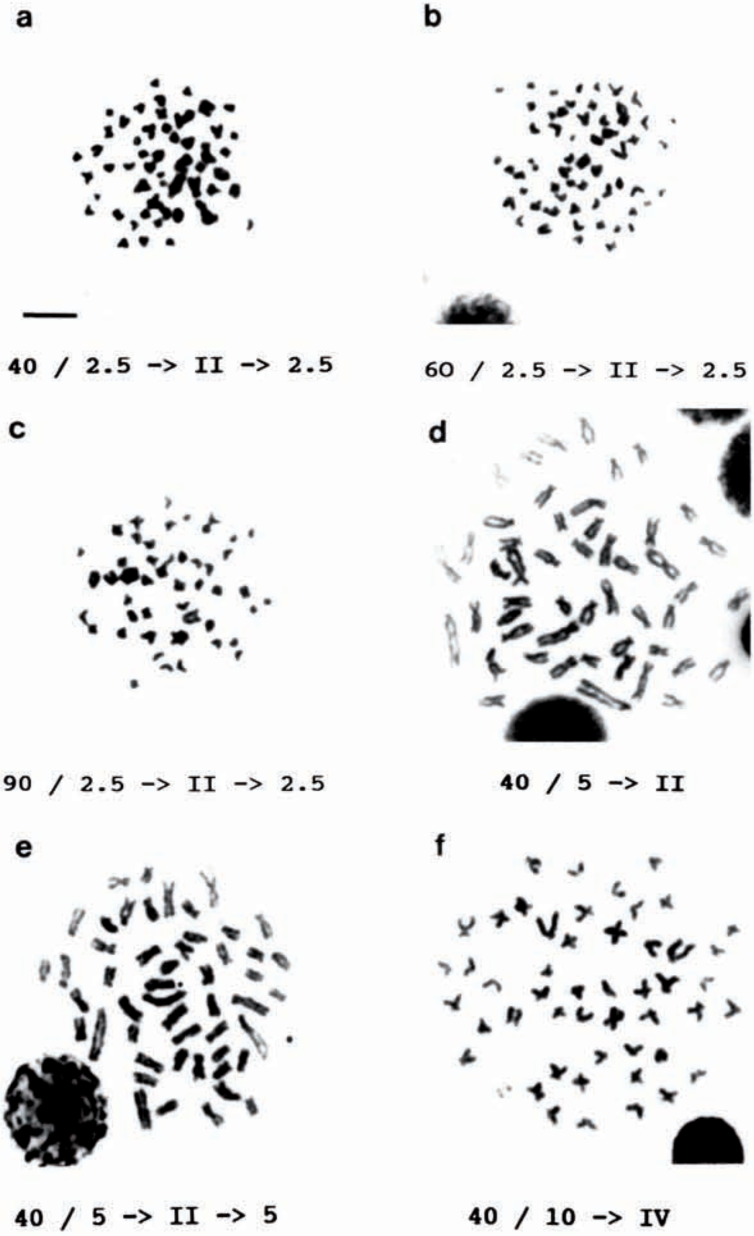
Slides were cleaned and prepared according to the protocol of Stanyon (pers. comm.), i.e. slides were put for 20 mn in a solution of 99 ml of absolute ethanol with 1 ml of HCl, then carefully wiped off with paper tissue just before laying down one drop of suspension covered by one drop of fresh fixative to the dry slide. Chromosomes were conventionally Giemsa stained.

RESULTS AND DISCUSSION

Metaphase plates of *S. glanis* and *S. erythrophthalmus* shown in figure 1 demonstrate the effects of different volumes of hypotonic solution using two different prefixation procedures.

The cells hypotonized in 2.5 ml of hypotonic solution and then prefixed with the same volume of fixative always displayed a bad spreading of chromosomes, irrespective of the hypotonic treatment duration. This is clearly shown in figure 1 a, b, c where hypotonization times were 40, 60 and even 90 mn respectively. On the other hand, good spreading of metaphase cells with clearly visible chromosome morphology was obtained when using volumes of 5 or 10 ml of hypotonic solution regardless of prefixation procedure used (Fig. 1d, e, f).

This observation led us to the conclusion that bad spreading of chromosomes was not related only to the type of prefixation procedure but to the volume of hypotonic solution itself. Thus there obviously seems to exist a limit ratio between the number of cells and the amount of hypotonic solution, i.e. amount of water available for a correct cell swelling. So, once the correct duration of hypotonic treatment has been established (i.e.



a 40 / 2.5 -> II -> 2.5

b 60 / 2.5 -> II -> 2.5

c 90 / 2.5 -> II -> 2.5

d 40 / 5 -> II

e 40 / 5 -> II -> 5

f 40 / 10 -> IV

Fig. 1. - Metaphase plates of *Silurus glanis* (a, b) and *Scardinius erythrophthalmus* (c, d, e, f). Below each photo are successively given duration of hypotonic treatment (in mn); volume of hypotonic treatment (in ml); volume of prefixation 1 (in drops, latin characters); volume of prefixation 2 (in ml). All metaphase plates are shown with the same magnification. Scale bar (a) represents 5 µm.

40 mn for the two species studied) the problem is undoubtedly related to the size of removed tissue from which the suspension is prepared. This has been already suggested by Klinkhardt (1991). We found that a minimum volume of 5 ml of hypotonic medium for a 0.1 g piece of kidney, enables regularly good chromosome spreading. This finding may contribute to explain the failures encountered by many fish cytogeneticists who often tend to utilize as much material as possible when preparing their cell suspensions. Moreover, if, in published protocoles for preparing fish chromosomes, the hypotonic medium volume can be sometimes indicated, the corresponding amount of tissue to be used is nearly never quantified.

We believe that such a technical detail will improve the quality of chromosomal preparations in order to enable the application of most sophisticated procedures (such as serial banding techniques or in situ hybridization).

Acknowledgements.- This work is a part of CNRS GDR 1005 program and was partly supported by le Ministère de la Recherche et de la Technologie (C. O.-C.) and le Muséum national d'Histoire naturelle (P. R.).

REFERENCES

GOLD J.R., LI Y.C., SHIPLEY N.S. & P.K. POWERS, 1990. - Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. *J. Fish Biol.*, 37: 563-575.

HOLMQUIST G.P. & M.A. MOTARA, 1987. - The magic of cytogenetic technology. *In:* Cytogenetics (Obe G. & A. Basler, eds), pp. 30-47. Springer-Verlag, Berlin-Heidelberg.

KLINKHARDT M.B., 1991. - A brief comparison of methods for preparing fish chromosomes: an overview. *Cytobios*, 67: 193-208.

THORGAARD G.H. & J.E. DISNEY, 1990. - Chromosome preparation and analysis. *In:* Methods for Fish Biology (Schreck C.B. & P.B. Moyle, eds) pp. 171-190. American Fisheries Society, Bethesda, USA.

Reçu le 18.06.1992.
Accepté pour publication le 09.07.1992.